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# EXPOSURE TO HEPATITIS C VIRUS

# EARLY IN LIFE

## -EPIDEMIOLOGICAL AND IMMUNOLOGICAL ASPECTS

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Karolinska Institutet

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# Exposure to hepatitis C virus early in life
## – Epidemiological and Immunological aspects

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**Stockholm 2018**

“Medicine is a science of uncertainty and an art of probability”

*William Osler*

In memory of Brigitta Omazic,
My mentor in science and life

To Fredrik, Vendela and Vincent

# ABSTRACT

Hepatitis C virus (HCV) is a blood borne virus affecting the liver. Globally, it is estimated that approximately 6 million children live with HCV infection today. The main transmission route in children today is vertical transmission. Before the introduction of blood donor screening in 1992, blood transfusion was an important source of infection. The aim of this thesis was to study epidemiological and immunological aspects of exposure to HCV early in life, with specific emphasis on transmission routes.

In **study I** we investigated blood transfusions in the neonatal period as transmission route in chronic HCV infection. We included 230 patients born 1960-75 with chronic HCV infection. Of those, 98 had unknown and 128 had known transmission route. Four out of 230 (1.7 %) had received blood transfusion in the neonatal period. None were aware of their transfusion history at the time of diagnosis. Three patients underwent successful antiviral treatment.

In **study II** the prevalence of HCV infection and HCV testing as well as infection outcome was studied in a cohort of 676 childhood cancer survivors in Stockholm. The prevalence of HCV testing was 34 % (233/676) for the whole cohort. Eleven out of 233 (5 %), were HCV-RNA positive, which is 10 times higher than the Swedish population prevalence. A comparison of the effectiveness of screening methods showed that active tracing screening was far more effective in terms of test uptake ($p < 0.001$). The majority of the infected patients underwent successful antiviral treatment.

In **study III** we investigated the impact of IL28B genotype on vertical HCV transmission. IL28B genotype is known to be important for spontaneous clearance and interferon treatment response. We included 59 mothers with chronic HCV infection and 123 children (47 infected and 76 uninfected) born of HCV infected mothers. Neither the mothers', nor the children's IL28B genotype was associated with the risk of vertically transmitted HCV (HCV-VT).

In **study IV** HCV specific T cell responses were studied in children born of mothers with chronic HCV infection. HCV specific T cell responses were investigated by IFNγ production and proliferation upon stimulation with viral antigens *in vitro*. We found an HCV specific T cell response in 73 % (11/15) of the mothers, 67% (6/9) of infected children and 56 % of uninfected/exposed (18/32) children. The two exposed pediatric groups had a significantly higher proportion of T cell responders compared to controls ($p< 0.02$). We detected HCV specific T cell responses already in exposed newborns, suggesting a prenatal exposure to viral antigens.

In conclusion, low awareness and prevalence of HCV testing in pediatric transfusion risk groups in combination with high HCV prevalence justifies active tracing screening of risk groups. IL28B genotype is not associated with HCV-VT, suggesting that other immunological factors need to be investigated. A high proportion of exposed but uninfected children have HCV specific T cell responses, suggesting that prenatal exposure to viral antigens might be more common than previously thought.

# LIST OF SCIENTIFIC PAPERS

I. **Einberg AP**, Lindh G, Hökeberg I, Papadogiannakis N, Fischler B. Neonatal blood transfusion as transmission route in chronic hepatitis C. Transfusion. 2014; 54:1366-70.

II. **Psaros Einberg A**, Ekman A, Söderhäll S, Millbourn C, Lindahl K, Harila-Saari A, Fischler B. High prevalence of chronic Hepatitis C virus infection among childhood cancer survivors in Stockholm, Sweden. In manuscript.

III. **Psaros Einberg A**, Duberg AS, Filipovich O, Nyström J, Zhirkov A, Brenndörfer ED, Frelin L, Rukoiatkina E, Lobzin Y, Sällberg M, Fischler B, Lutckii A. Lack of association between interleukin 28B polymorphism and vertical transmission of hepatitis C. J Pediatr Gastroenterol Nutr 2017;65(6):608-12

IV. **Psaros Einberg A**, Brenndörfer ED, Frelin L, Hallberg L, Sällberg M, Fischler B. Neonatal exposure to hepatitis C virus antigens in uninfected children Born to infected mothers. J Pediatr Gastroenterol Nutr 2018;66(1):106-11.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 5BdrU | 5-bromo-2′-deoxyuridine |
| CI | Confidence interval |
| CMI | Cell mediated immune |
| CPM | Counts per minute |
| DAA | Direct acting antiviral |
| EIA | Enzyme immune assay |
| ELISA | Enzyme-linked immunosorbent assay |
| ELISpot | Enzyme-linked immunospot |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HCV-gt | Hepatitis C virus genotype |
| HCV-VT | Hepatitis C virus-vertically transmitted |
| HIV | Human immunodeficiency virus |
| HLA | Human leucocyte antigen |
| IDU | Injection drug use |
| IFN | Interferon |
| IFNL3 | Interferon lambda 3 |
| IL28B | Interleukin 28B |
| ISG | Immune stimulating genes |
| JAK-STAT | Janus kinas-signal transducer and activator of transcription |
| KIR | Killer-cell immunoglobulin-like receptor |
| LKM-1 | Liver kidney microsomal antibody type 1 |
| NANB | Non-A Non-B |
| NS2 | Non-structural protein 2 |
| NS3 | Non-structural protein 3 |

| | |
|---|---|
| NS4A | Non-structural protein 4A |
| NS4B | Non-structural protein 4B |
| NS5A | Non-structural protein 5A |
| NS5B | Non-structural protein 5B |
| P7 | Protein 7 |
| PBMC | Peripheral blood mononuclear cells |
| PCR | Transcriptase polymerase chain reaction |
| PWID | People who inject drugs |
| RIBA | Recombinant immunoblot assay |
| RNA | Ribonucleic acid |
| SFU | Spot forming unit |
| SI | Stimulation Index |
| SNP | Single genetic polymorphism |
| SVR | Sustained viral response |
| WHO | World Health Organization |

# 1 INTRODUCTION

Hepatitis C virus (HCV) infection is a blood borne disease affecting the liver. Chronic HCV infection is an important global health issue, affecting an estimated 71 million people worldwide (1). Among them, around 6 million are children (2). The majority of HCV infected individuals are unaware of their infection status (1, 3). Chronic HCV infection is usually asymptomatic the first 20 years, but might eventually progress into liver cirrhosis and hepatocellular carcinoma (HCC) (3). The introduction of highly effective and safe direct acting antiviral (DAA) treatment has revolutionised the treatment outcome of HCV infection, which is now considered a relatively easy-to-treat disease. The World Health Organisation (WHO) has set a goal of eliminating viral hepatitis as a public health threat by the year of 2030 (1). To reach this goal, up scaling of screening methods to find yet undiagnosed patients and prevention strategies to reduce viral transmission are needed.

This thesis aims to explore the past and present main transmission routes of HCV in children, namely vertical transmission from mother to child during pregnancy or delivery and previous blood transfusions. We study the association between immunological factors and the risk of vertical transmission and the HCV prevalence and screening outcomes of paediatric transfusion risk groups.

# 2 BACKGROUND

## 2.1 HISTORY

In the 1970s a previously not described post transfusion viral hepatitis infection was suggested and named non-A, non-B (NANB) hepatitis, since it lacked serological markers of both hepatitis A and B (4). However, it took until 1989 before the new virus, named hepatitis C, was isolated and characterized. Isolation was performed by a novel, blind immunoscreening method in which antibodies derived from a clinically diagnosed NANB hepatitis patient were used to identify a cDNA clone encoding an epitope within the hepatitis C virus. (5). Serological tests were developed and became available in Sweden by 1990. Blood donor screening for hepatitis C by second generation antibody testing was introduced in Sweden by January 1st 1992. Interferon treatment was available already in the 1980's, later with the addition of ribavirin (6). This interferon-based injection therapy was only moderately effective and had serious side effects. Intensive virological research led to the development of the highly effective oral direct-acting antiviral (DAA) treatment introduced in 2011 (7-9).

## 2.2 EPIDEMIOLOGY

The World Health Organization (WHO) has recently, in the "World hepatitis report 2017", recalculated the global burden of chronic hepatitis C infection. The number of individuals assumed to be infected was thus reduced from previously 170 million people to 71 million people; the new number is based on updated estimations (1, 2). The most important reason for this big difference in prevalence is that the new estimation is based on viremic infections (i.e. positive HCV RNA) instead of serologic (HCV-antibody) status which was used previously (1) . The new estimate of 71 million people constitutes a global HCV prevalence of 1 %. It is estimated that approximately 6 million children under the age of 15 years have viremic HCV infection, which gives a prevalence of around 0.3% (2). Worldwide, in 2015 a total of 1.75 million new cases of chronic hepatitis C were diagnosed and the most common routes of HCV transmission were injection drug use (IDU) and unsafe health care procedures. It is estimated that 2.3 million people with HCV also are co-infected with human immunodeficiency virus (HIV).

The global incidence rate of HCV infection has decreased during many years, but in the US a 170 % increase in incidence rate of acute HCV infection among young people in nonurban counties was seen from 2006-2012, with the steepest increment in 2011-2012 (10). The explanation for this alarming trend is the increasing drug use among young people in the US. The actual incidence of acute HCV infection (see below for details) is hard to calculate since the infection is mainly asymptomatic. Chronic HCV infection is unevenly distributed around the world with highest prevalence in Eastern Europe, Central Asia and Egypt (Fig. 1). The morbidity and mortality rates caused by chronic HCV infection have increased since year 2000 and it is estimated that around 400 000 people die from HCV related causes every year.

Unlike Malaria, Tuberculosis and HIV, the mortality trend for viral hepatitis is increasing (11).

In Sweden, the prevalence of anti-HCV among adults is calculated to be around 0.5 % (12). The majority of these patients was born in 1950-1960 and they were infected mainly by IDU during the 1970s to 1980s. In Sweden, notification of HCV infection is mandatory to report to the Public Health Agency of Sweden by clinicians and laboratories according to the Communicable Diseases Act. Around 2000 new cases of chronic HCV infection are reported every year (13).

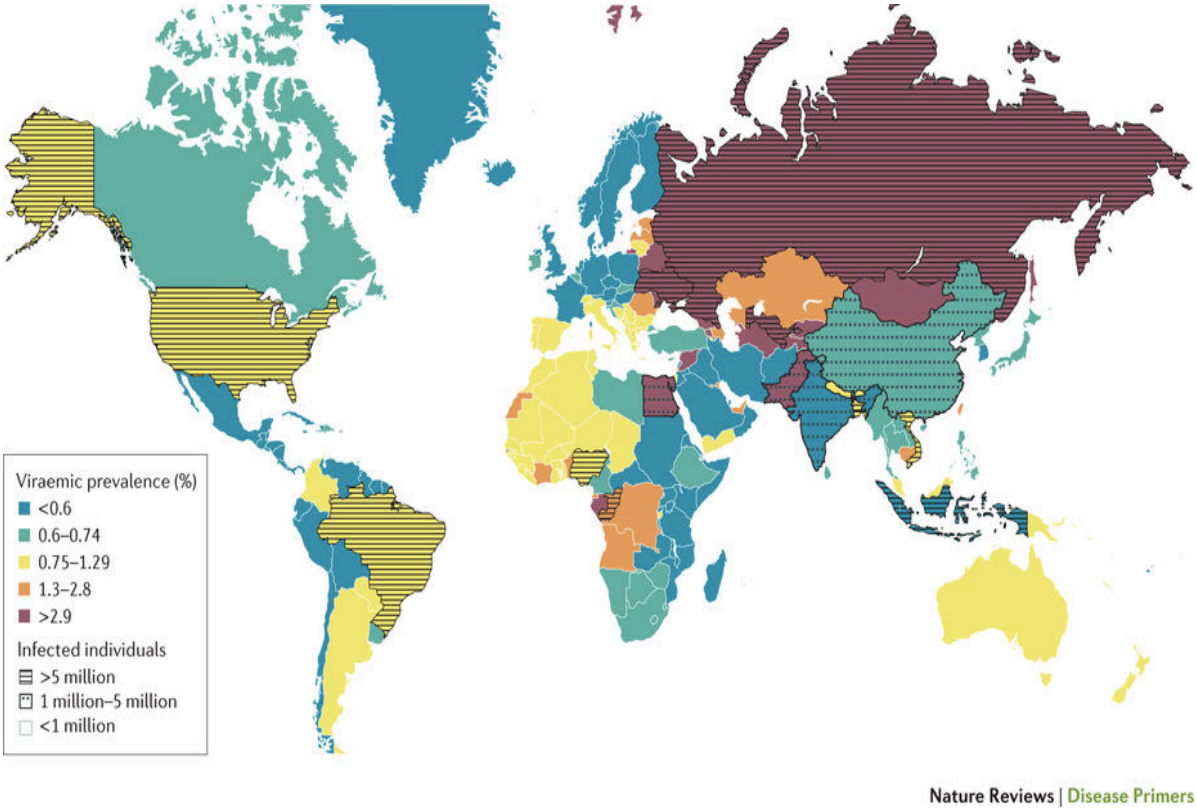



**Figure 1.** *Global prevalence of hepatitis C virus infection in 2017.*

*Reprinted by permission from Springer Nature: Nature Reviews Disease Primers, Hepatitis C virus infection, Michael P. Manns et.al, © 2017.*

## 2.3 TRANSMISSION ROUTES AND HCV SCREENING

Hepatitis C virus infection is a blood-borne disease and globally the most common routes of infection are injection drug use (IDU), blood product transfusions, contaminated health care equipment, sexual transmission and mother-to-child transmission during pregnancy or delivery (14).

### 2.3.1 Blood transfusions and look-back screening

In countries where general blood donor screening for HCV was introduced in the early 1990'es, infection through contaminated blood products has almost completely vanished as transmission route. However, in some low-income countries, transfusion of blood products and contaminated health care equipment is still a common source of infection (1). Many people living with chronic hepatitis C today were infected by contaminated blood through transfusions before 1992. Nationwide screening campaigns and so called look-back studies of blood product recipients have been carried out in some countries in order to detect and treat HCV carriers (15, 16). There are two types of look-back studies; **general look-back studies** that aim at a specific risk group, and **targeted look-back studies** that proceed from a known donor of HCV contaminated blood. Unawareness of previous blood transfusion during childhood is common. Children, with a long life expectancy, run a higher lifetime risk of HCV related complications than adults.

The Swedish National Board of Health and Welfare launched a national hepatitis C screening campaign in 2007 (17). Individuals who recalled a blood product transfusion in the years 1965-1991, were by advertisements in media recommended to take a test for anti-HCV. Three specific paediatric risk groups with a high estimated prevalence of blood transfusions were identified:

1. Patients treated for prematurity, in particular those who had undergone neonatal exchange transfusion.
2. Patients treated for childhood cancer.
3. Patients who had undergone open heart surgery during childhood.

By the end of year 2010, 65 000 subjects in the Sweden had been tested for HCV within the screening campaign, of whom 0.9 % were anti-HCV positive and 0.6 % HCV-RNA positive (17). The majority was born in the 1930-50's, i.e. presumably not belonging to any of the three specific pediatric risk groups. A large group of women was found to be infected by blood transfusion during childbirth. For comparison, a summary of the screening campaign in the region of Västra Götaland demonstrated a prevalence of HCV viremia of 0.8 % (15).

General look-back studies on recipients of neonatal blood transfusions have shown a prevalence of HCV viremia of around 1-3 % (18, 19). However, an Italian study of recipients of neonatal micro transfusions during 1968-74 reported a very high prevalence of 30 % HCV viremia (20). The same group did later a targeted look-back study on 31 recipients of mini transfusions given at birth in 1968 from a common infected donor and found 58 % to be anti-HCV positive and 52 % also HCV-RNA positive, none of them were aware of their transfusion history (21). Similar results were found in a Swedish targeted look-back study from 1996 on transfusion recipients of infected donors , with 93 % anti-HCV positive and 59 % HCV-RNA positive (22). Studies have confirmed that at least half of neonatal transfusion recipients are unaware of their previous transfusion history (19, 23). Vogt et al. studied

patients who underwent cardiac surgery during childhood before 1991and found a prevalence of 8 % positive HCV-RNA (24).

Previous international studies of childhood cancer survivors have reported an HCV-RNA prevalence of around 6-40 % in this risk group, depending on which malignancies that were included (25-27). An increased risk of HCV related complications and a more advanced liver disease due to the additional effect of cytotoxic and immunosuppressive treatment have been shown in some studies, while others claim no additional effect (27-29).

### 2.3.2 Vertical transmission (Mother-to-child transmission)

Vertical transmission of HCV (HCV-VT), also called mother-to-child transmission (MTCT), during pregnancy or delivery is nowadays the most common route of HCV transmission in children. The risk of HCV-VT in children of HIV negative mothers is estimated to be around 5 % (range 1-10 %), (Table 1) (30, 31). HIV co-infection doubles the risk of HCV-VT (Table 1). Two early Swedish studies on HCV-VT showed a low rate of transmission (32, 33).

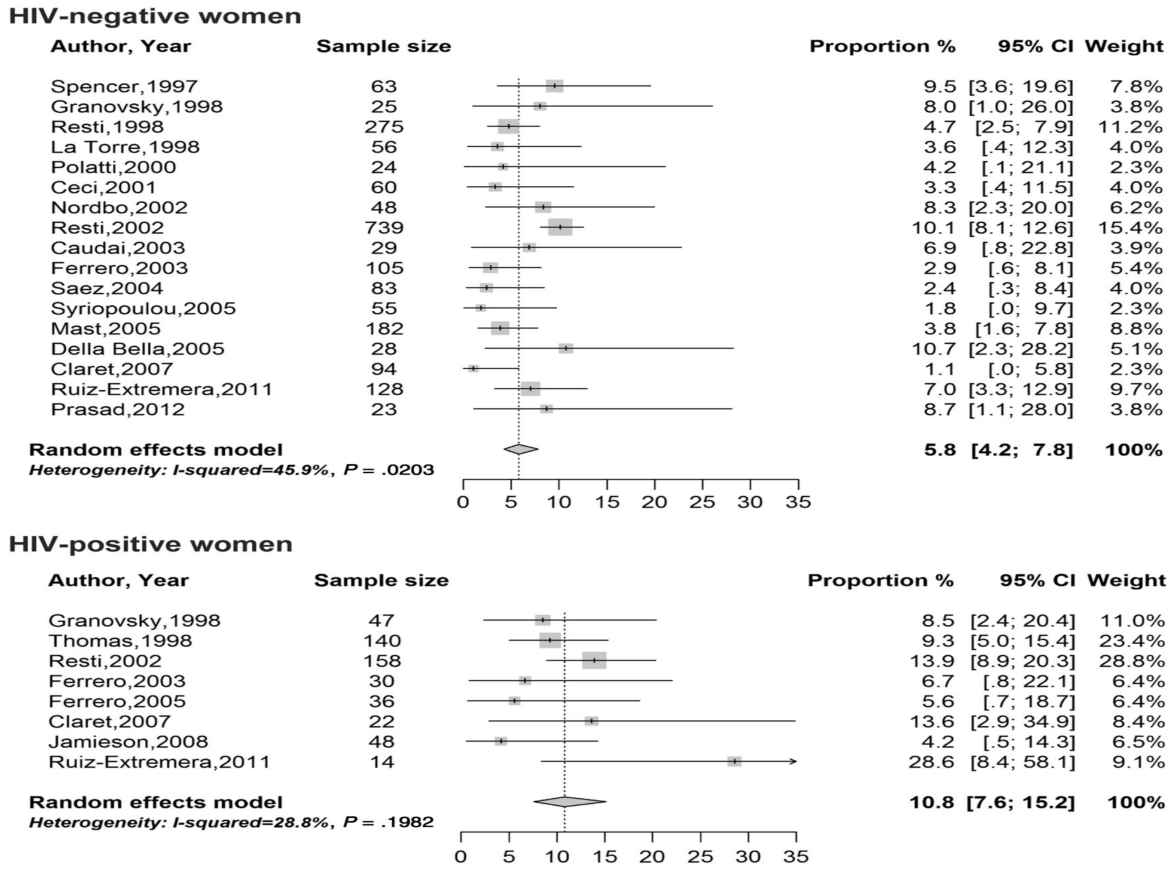



**Table 1.** *Pooled estimates of risk of hepatitis C virus (HCV) vertical transmission among children ≥18 months born to HCV antibody–positive and RNA-positive mothers, by maternal HIV serostatus. With permission from Oxford University Press. Benova et al. Vertical Transmission of Hepatitis C Virus: Systematic Review and Meta-analysis. Clin Infect Dis. 2014;59(6):765-773.*

The exact time point of transmission is difficult to determine, but around 1/3 of all cases are thought to occur during pregnancy and the rest during delivery (34). Potential risk factors for HCV-VT have been investigated in numerous studies. Clinical factors associated with an increased risk of HCV-VT are co-infection with HIV, high maternal viral load, prolonged rupture of membranes and possibly fetal scalp monitoring during labor (35-39). One multicenter study described a two times higher risk of vertical HCV infection in girls compared to boys, the reason for this finding is unclear (40). Delivery mode or breast feeding have not been shown to influence the risk, and mothers with HCV infection are not discouraged from vaginal delivery or breastfeeding (38). All children of HCV infected mothers are recommended to be tested for anti-HCV at the age of 18 months when passively transferred maternal antibodies have disappeared. No country has implemented general antenatal HCV screening of pregnant women. Only pregnant women considered to be at risk (e.g. previous or present drug use, blood transfusion before 1992) are currently tested. The reason for not including HCV in antenatal screening programs is lack of interventions to prevent HCV-VT. However, the group of HCV infected women that lack known risk factors is missed if only patients from risk groups are included in antenatal screening (41).

Despite viral exposure of the fetus during delivery, the rate of HCV-VT is low compared to the vertical transmission rate of other viral infections, such as HIV and hepatitis B virus (HBV) (42). The reason for the comparatively low risk of HCV vertical transmission remains unclear, but various immunological factors have been suggested to play a role, for example human leukocyte antigen (HLA) genotype, HCV-specific T cell responses and HCV neutralizing antibodies (43-46). Difference in maternal HCV genotype does not seem to influence the risk of HCV-VT (38).

### 2.3.3 Injecting drug use

The most common source of HCV infection in adults is injecting drug use (IDU) and sharing of needles. The term PWID (people who inject drugs) is preferred when referring to the subjects as a group. It is estimated that 8 % (5.6 million people) out of the 71 million HCV infected people are active drug users (1). PWID accounts for 23 % of new HCV cases worldwide (1). In Sweden, around 39 % of persons notified with HCV infection report IDU as transmission route (13). A recent study of PWID in Stockholm showed an 82% prevalence of anti-HCV and 76% prevalence of HCV-RNA (47). In Stockholm the first needle exchange program was introduced as late as in 2013. Needle exchange programs for PWID need to be combined with HCV treatment and opioid substitution treatment to reduce the HCV transmission rate in this group (48, 49). Efforts to screen and treat this risk group have been of various success, mainly due to lack of reachability. The risk of reinfection is higher in PWID, but nevertheless ability to reach this group that otherwise has little interaction with health care services, remains a key factor to control the HCV epidemic (47). Screening and treating HCV infected prisoners, a group with high proportion of PWID have been effective in many countries (50).

### 2.3.4 Other transmission routes

A large proportion of HCV infections are notified as unknown transmission route. In Sweden, approximately 45 % report unknown transmission route when diagnosed with HCV infection (13). Studies have shown that careful risk factor screening can reduce this proportion. A remote history of IDU was the true source of infection in almost all cases with reported unknown transmission route in a study by Flamm et al. (51). The contribution of other possible transmission routes to the burden of HCV infection is small but not negligible. Transmission of HCV has been described in tattooing (52), intranasal drug use (53), sexual contacts (with a higher risk for men who have sex with men (54)) and (in low-income countries) the use of unsterilized health care equipment (1). Unawareness of previous blood transfusions, especially if given in the neonatal period, might also explain some of the cases with unknown transmission route (20, 21).

## 2.4 HEPATITIS C VIROLOGY

HCV is a single stranded enveloped RNA virus belonging to the Flaviviridae family. Its genome consists of 9600 nucleotides encoding a large polyprotein of about 3000 amino acids. The polyprotein is cleaved by host and viral proteases into 10 known proteins; three structural (core and envelope 1 and 2) and seven non-structural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Fig. 2). The HCV polymerase is prone to error reading, which gives rise to a high mutation rate and genetic variability of the virus. This is one of many viral mechanisms for evasion of the host immune system. Currently there are 7 known HCV genotypes with different geographic distribution. Until now different treatment modalities were needed for specific genotypes.

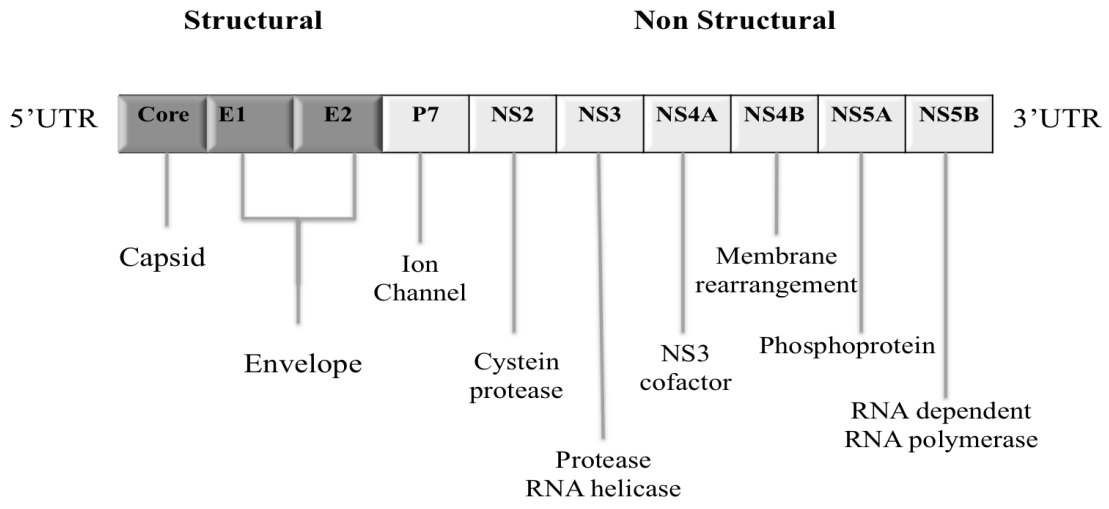



**Figure 2.** *The HCV polyprotein and its functional protein products*

## 2.5 HEPATITIS C IMMUNOLOGY AND VIRAL EVASION

The virus infects predominantly hepatocytes in the liver. Within a week after infection viremia can be detected as positive HCV RNA in peripheral blood. The innate immune system recognizes HCV and induces an early interferon (IFN) response (55). However, the virus manages to attenuate the Janus kinas-signal transducer and activator of transcription (JAK-STAT) pathway which results in impaired host IFN production (56, 57). As a consequence of failure of the innate immune system to control viral replication, the HCV RNA levels increase dramatically the first weeks after infection. The adaptive immune response, including neutralizing antibodies and HCV specific T cells, is activated about 4-8 weeks after infection (58). Patients with acute HCV show broad HCV-specific T cell responses against different viral epitopes (58). This broad immune response is lost over time in the case of chronic infection, and viral escape mutations emerge, that contribute to viral evasion of the host immune system (59). The effector cells in chronic HCV infection are mainly CD8+ T cells, which are dependent on help from CD4+ cells. In chronic HCV infection the CD4+ cells are deficient which contributes to exhaustion and dysfunction of CD8+ effector cells that are unable to eradicate the virus and chronicity is established (55). Spontaneous resolution occurs in about 20 % of acutely infected patients and is dependent on an intense and broad IFN-γ response, mediated by HCV specific T cells. The humoral response in HCV infection is unable to clear the infection; however neutralizing antibodies might yield some degree of protection (60, 61).

Protective immunity with decreased duration of infection upon reinfection has been shown in humans, although sterilizing immunity has not been proven (62). Studies on HCV specific T cell responses in individuals at high risk of HCV infection have shown that individuals with strong and broad responses might be partially protected from chronic infection, and that reinfection in T cell responders yielded a shortened viremia period (63-67). Chimpanzee rechallenge studies have demonstrated that HCV specific T cell responses were associated with spontaneous clearance and protection from chronic reinfection (68, 69). It has been hypothesized that children who are exposed to HCV antigens in utero might be endowed with some sort of protective immunity (44). Previous in vitro studies have shown that vertical exposure to HCV induces virus-specific cell-mediated immune (CMI) responses in uninfected children of mothers with HCV infection (44, 70).

Due to insufficiency of the innate and adaptive immune system in combination with viral immune evasion strategies the infection becomes chronic with risk of developing liver fibrosis in about 75-80 % of infected patients (Fig. 3) (71, 72).

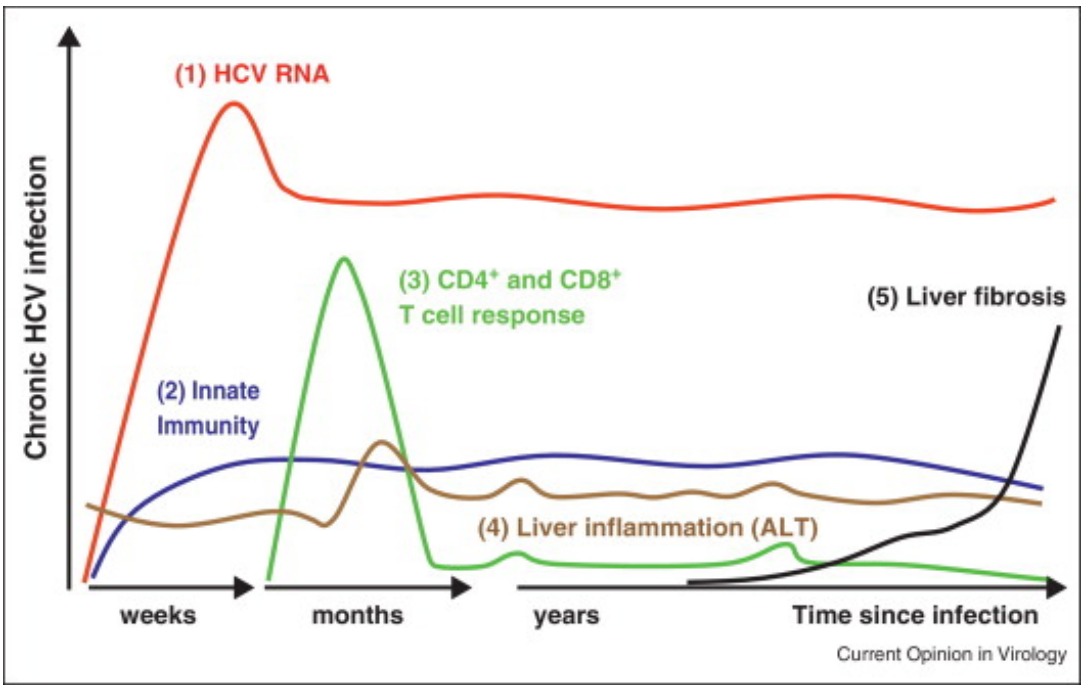



**Figure 3.** *Kinetics of subsequent processes: first viral replication (HCV RNA), second innate immunity, third HCV-specific CD4$^+$ and CD8$^+$ T cell responses, fourth liver inflammation (ALT) and fifth liver damage (fibrosis). Reprinted from Curr Opin Virol 2013;3(4):461-7, Claassen et al, Role of T cell immunity in hepatitis C virus infections, with permission from Elsevier*

## 2.6 IL28B SINGLE GENETIC POLYMORPHISM

In 2009, Ge et al. described that single genetic polymorphism (SNP), near the gene for Interleukin 28B (IL28B) on chromosome 19 (SNP *rs12979860*) is associated with better treatment response in interferon-based treatment (73). The IL28B gene encodes type III interferon, IFN-λ3 (IFNL3). IL28B has recently been renamed IFNL3. It was also shown that spontaneous viral clearance was strongly associated with the favourable IL28B genotype C/C (74). The favourable effect of the C allele is particularly strong in patients infected with HCV genotype 1, and slightly less pronounced regarding viral genotype 2 and 3 (75, 76). Results from paediatric studies on spontaneous clearance and IL28B genotype are in line with the adult observations (77-79).

The product of the IL28B gene, the IFNL3, mediates its effect on treatment results and spontaneous clearance by activating the JAK-STAT pathway. The activation induces interferon-stimulating genes (ISGs) that have antiviral activity. Presence of the unfavourable T allele results in less IFNλ3 expression (78). IL28B/IFNL3 genotype has, in the era of DAA treatment, no longer any clinical implications on treatment decisions. However, it is still relevant for the rate of spontaneous clearance.

## 2.7 DIAGNOSIS OF HEPATITIS C VIRUS INFECTION

The HCV antibody test is used to screen for hepatitis C infection and the HCV RNA test to confirm or exclude ongoing infection. HCV antibodies are analysed by enzyme immune assay (EIA), where recombinant antigens are used to capture circulating antibodies (80). To confirm a positive EIA test, a recombinant immunoblot assay (RIBA) is used (81). Reverse transcriptase polymerase chain reaction (PCR) and real time PCR is used to detect and quantify HCV RNA (82).

HCV RNA can be detected in blood within a week after infection, while HCV antibodies are developed 6-8 weeks later (83). HCV antibodies remain positive for many years in patients who have cleared the infection spontaneously or after treatment (84). The diagnosis of acute hepatitis C infection is made by detection of HCV RNA in blood, and if still detectable six months later the infection has developed into chronic hepatitis C.

The definition of spontaneous clearance is two negative HCV RNA tests taken at least 12 weeks apart. One negative HCV RNA test is not sufficient to declare spontaneous clearance since transient suppression of viremia has been demonstrated (85). Approximately 25 % of adults spontaneously clear the acute infection, while 75 % develop a chronic infection (86). The majority of patients who spontaneously clear the infection do that within a period of six months from infection; only 11 % clear the infection later (86). Factors associated with spontaneous HCV clearance are female gender, low viral load and young age at infection, viral genotype 1 and IL28B genotype C/C (86, 87).

To determine the amount of HCV related liver fibrosis and inflammation, a percutaneous liver biopsy can be performed. Histologically, the stage of liver fibrosis as well as the grade of inflammation is measured using different scoring systems. In Sweden the Ludwig and Batts score is the most commonly used, which grades liver inflammation 0-4 and stages liver fibrosis 0-4 and (88). Liver biopsy is not a risk free event and serious complications such as massive bleeding do occur occasionally (89). In recent years, transient liver elastography, which is a non-invasive ultrasound based method to measure liver stiffness or elasticity, has been developed and is now commonly used to evaluate the degree of liver fibrosis (90).

## 2.8 NATURAL HISTORY OF HCV INFECTION IN ADULTS

The acute infection is short-term and usually asymptomatic but around 10 % develop hepatitis symptoms such as jaundice, fatigue and nausea (91). Liver transaminases are usually elevated.

The chronic infection is usually life-long and asymptomatic. However, it leads to progressive liver inflammation and silent development of liver fibrosis resulting in end-stage liver disease 20-30 years later in 20-50 % (71, 72). Around 50 % of those infected are unaware of their HCV status and it is estimated that only 20 % of those infected have been diagnosed (1, 92). The first symptoms might in some cases, be related to already established liver cirrhosis such

as jaundice, ascites and gastrointestinal bleeding. The risk of developing hepatocellular carcinoma (HCC) is clearly elevated in patients with HCV related liver cirrhosis, with an annual HCC rate of around 1-4 % in Europe and USA (93, 94). A Swedish study concluded a 7 % absolute risk of developing primary liver cancer within 40 years of HCV duration (95). Patients with HCV related cirrhosis should thus participate in HCC surveillance programs including regular ultrasound scanning. The rate of HCV related liver fibrosis varies between individuals. Factors associated with a higher rate of fibrosis development are high age at infection, duration of infection, male gender, alcohol consumption, obesity, co-infection with HIV and viral genotype 3 (71, 96-98). There are contradicting findings regarding the impact of IL28B genotype on development of liver fibrosis (99, 100). Hepatitis C infection can also give rise to extrahepatic manifestations such as cryoglobulinemia, membranous glomerulonephritis and lymphoproliferative disease (101).

## 2.9 NATURAL HISTORY OF HCV INFECTION IN CHILDREN

In children HCV infection is considered to be less progressive, maybe due to lack of certain adult risk factors such as alcohol consumption. Most children have no clinical symptoms at all during childhood even though moderately elevated liver transaminases are common. However, some children and adolescents do develop an advanced liver disease with progressive fibrosis and even HCV related cirrhosis (102-104).

The most common route of HCV infection in children nowadays is mother-to-child transmission during pregnancy or delivery (105, 106). The rate of spontaneous clearance after perinatal transmission is calculated to be around 20 % and the median time of clearance is 15 months after infection (103). Factors associated with spontaneous viral clearance in vertically infected children are hepatitis C specific IFNγ immune response, IL28B genotype C/C and viral genotype 3 (70, 102, 107). There are conflicting data concerning the impact of abnormal ALT levels on the rate of spontaneous clearance in children (108-110). The high viral mutation rate gives rise to wide HCV genome diversity within each infected individual, termed HCV quasispecies. Those quasispecies helps the virus evade the host immune system, leading to higher risk of chronicity. The diversity is largest within the E2 region, which is also under selective pressure from HCV neutralizing antibodies (111). It has been shown that HCV-VT infected children have less diverse viral E2 region compared to their mothers which theoretically might contribute to higher rates of spontaneous clearance in young children, also suggesting that transient viremia might be more common than previously thought during the first 18 months of life (112).

Extrahepatic manifestations of chronic HCV infection are rare in children, but a higher percentage of autoantibodies have been described. Presence of liver kidney microsomal antibody type 1 (LKM-1) was found in 10 % of children with chronic HCV infection and was associated with a higher degree of liver fibrosis (113, 114). Children with chronic hepatitis C

are recommended to be followed by yearly clinical check-ups including blood tests and assessment of liver fibrosis.

## 2.10 HEPATITIS C TREATMENT

Pegylated interferon in combination with ribavirin was the standard of care treatment since the 1990s (6). This treatment was not very effective and had multiple side effects. Since 2011, the development of targeted direct acting antiviral (DAA) therapy has revolutionized the treatment of HCV infection (9, 115) . The interferon free combinations introduced in 2014 cure chronic HCV infection in > 95 % of patients, regardless of viral genotype, fibrosis stage or co-infection with HIV (116) . Cure, meaning sustained viral response (SVR), is defined as negative HCV-RNA 24 weeks after cessation of treatment. The new treatment has almost no or only mild side effects. DAAs target three proteins in the HCV life cycle: NS3/NS4A protease, NS5B polymerase and NS5A protein (Table 2).

Different combinations of DAAs are used for different genotypes and patient groups and new treatment combinations are approved frequently (Table 2). Recently pangenotypic treatments have been developed (117). The DAA treatment usually consists of 1-3 oral pills per day for 8-12 weeks in most cases and no longer than 24 weeks. This is in contrast to the older interferon based treatment, which had to be given as injections for up to 12 months.

DAA treatment has been shown to be as effective and safe in children as in adults and was recently made available for children with chronic HCV infection above 12 years of age in EU and US (118-120). Several pediatric DAA studies are ongoing. DAA treatment has revolutionized the management of chronic HCV infection and changed the view on this disease from hard- to easy- to treat. The treatment is still very expensive and at the moment only few countries can afford to treat all patients with chronic HCV. Treatment uptake is low even in some high-income countries. In Sweden only patients (including children) with fibrosis score F2-F4 were recommended DAA treatment from 2014. However, with reduction in prices the latest Swedish recommendations favour treatment for all chronically infected (121). In many low-income countries, IFN based treatment is still the most common option, however generic DAA treatment to a lower cost has recently become available, in fact also for children (122). Efforts have been made to develop a vaccine against HCV, but there is yet today no such vaccine available. The effectiveness of DAAs has encouraged WHO and many countries to set up goals to eliminating the disease as a major health threat by 2030 (1).

**Table 2.** *Available DAA treatment regimens in Europe by February 2018. DAA treatment should always involve a combination of at least two substances. Ribavirin can be added to all the combinations.*

| NS5B polymerase inhibitor | NS5A inhibitor | NS3/4A protease inhibitor | Genotype |
|---|---|---|---|
| Sofosbuvir | | | 1-6 |
| | | Simeprevir | 1,4 |
| | Daclatasvir | | 1-4 |
| Sofosbuvir[1] | Ledipasvir[1] | | 1, 4 |
| Sofosbuvir[2] | Velpatasvir[2] | | 1-6 |
| | Ombitasvir | Paritaprevir (+/- Ritonavir) | 1,4 |
| Dasabuvir | | | 1 |
| | Elbasvir[3] | Grazoprevir[3] | 1,4 |
| | Pibrentasvir[4] | Glekaprevir[4] | 1-6 |
| Sofosbuvir[5] | Velpatasvir[5] | Voxilaprevir[5] | 1-6 |

[1] Fixed combination Sofosbuvir + Ledipasvir; Harvoni®
[2] Fixed combination Sofosbuvir + Velpatasvir; Epclusa®
[3] Fixed combination Grazoprevir + Elbasvir; Zepatier®
[4] Fixed combination Pibrentasvir + Glekaprevir; Maviret®
[5] Fixed combination Sofosbuvir + Velpatasvir + Voxilaprevir; Vosevi®

# 3 AIMS

The overall aim of this thesis was to study epidemiological and immunological aspects of hepatitis C virus exposure early in life.

Specific aims were:

- To investigate the role of neonatal blood transfusions as the transmission route of HCV infection in adult patients and to describe the natural course in those presumably infected by neonatal blood transfusion (paper I).

- To investigate the prevalence of HCV infection and previous HCV testing among childhood cancer survivors in Stockholm and to screen those previously untested (paper II).

- To investigate if IL28B genotype was associated with risk of vertical transmission of HCV infection (paper III).

- To study the presence and relevance of HCV specific T cell responses in children born of mothers with chronic HCV infection (paper IV).

# 4 MATERIALS AND METHODS

## 4.1 PATIENTS AND STUDY DESIGN

Three different study populations were used. Paper III and IV included partly the same group of patients, where the Stockholm cohort was the same in both papers but in paper III patient cohorts from Örebro and St Petersburg were added.

### 4.1.1 Paper I

In this retrospective register-based cohort study, 255 adult patients born 1960-1975 with chronic hepatitis C virus infection and followed at Karolinska University hospital, Stockholm, Sweden were contacted by mail and invited to participate. In total, 230 patients were eligible for the study (Fig. 4). Maternity and/or neonatal medical records of all included patients were studied for the occurrence of a neonatal blood transfusion. Patients without a documented referral to a neonatal ward were considered not transfused since blood transfusions are given at neonatal units. The prevalence of previous neonatal blood transfusion was then estimated and compared between the patients with known versus unknown HCV transmission route. Clinical data on the natural course of HCV infection in patients transfused as neonates were collected from the hospital medical record system. Available liver biopsy specimens were re-evaluated by a pathologist who had no previous knowledge of the patients.

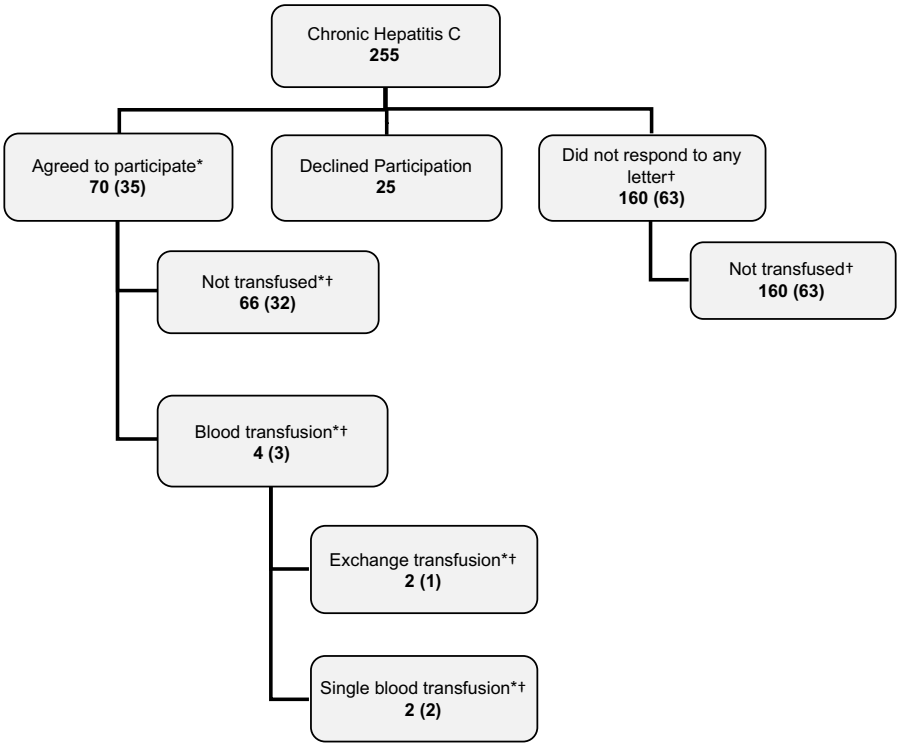



**Figure 4.** *All 255 patients eligible for study I, subdivided as participants, decliners, and nonresponders. *Maternity records collected and studied. †Neonatal records collected and studied. (n) = number of patients with unknown transmission route according to data in medical records.*

### 4.1.2 Paper II

This was a combined retrospective register-based and prospective screening study of adult childhood cancer survivors treated for malignancy in Stockholm before 1992. First, we investigated the prevalence of HCV infection and previous anti-HCV testing. Next, we screened the remaining and untested cohort for HCV infection. A clinical pediatric oncology register was used, containing 2171 patients who had undergone investigation and/or treatment at the Pediatric Oncology department at Karolinska University hospital, Stockholm, Sweden from 1921 to 1991. Patients with benign diagnoses, age above 18 years at initiation of therapy, deceased before 1992 and patients registered without a personal identification number were excluded. Patients in the included remaining cohort (n = 686) were then checked for existing notification of hepatitis C infection in the national register of hepatitis C cases at the Public Health Agency. The degree of liver disease and antiviral treatment outcome of patients diagnosed with HCV infection was analyzed. To investigate the prevalence of previous HCV testing, medical records as well as laboratory registers were analyzed for documented anti-HCV test.

In the second prospective part of the study, a cohort of 231 patients living in Stockholm County without a traceable and documented anti-HCV test were contacted by letters and invited to participate in the HCV screening study (Fig.7). Patients, who choose to participate in screening by returned written informed consent, were offered a blood test for anti-HCV free of charge at the nearest primary health care center. Two reminding letters were sent. Analysis was made of age, gender, malignancy diagnosis, time of treatment and HCV testing as well as results of anti-HCV screening.

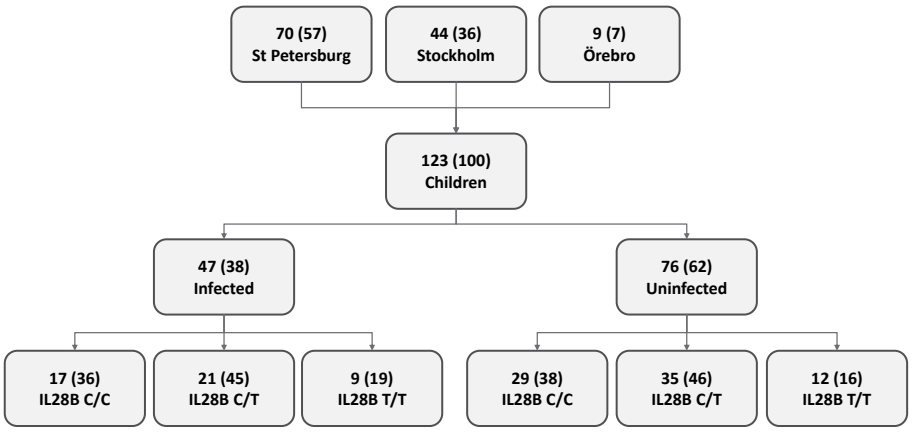
### 4.1.3 Paper III

This was a cross-sectional study of the Stockholm cohort used in paper IV, with the addition of two new cohorts from Örebro and St Petersburg. Patients were enrolled from three different pediatric departments at Karolinska University Hospital in Stockholm, Örebro University Hospital in Örebro, Sweden and Science Research Institute of Children's infections in Saint Petersburg, Russia. In total 59 HCV-RNA positive/HIV negative mothers and 123 children born to HCV-infected/HIV negative mothers were included and their stored plasma samples were analyzed for IL28B polymorphism (SNP *rs12979860*) and classified by the IL28B genotype (C/C, C/T and T/T) as well as by viral genotype. The 59 mothers gave birth to 70 children who were included together with another cross-sectional cohort of 53 children of HCV-RNA positive mothers where the maternal IL28B status was unknown (Fig. 5). Out of the included 123 children, 47 were vertically infected and 76 HCV exposed but uninfected. The patients from the Stockholm cohort were the same as in paper IV with the addition of 10 exposed but uninfected children and 5 infected mothers. Exposed children were enrolled when routinely tested for HCV antibodies at the age of 18 months. The 47 HCV infected children were enrolled in the study as patients followed at the pediatric

departments. Uninfected children were considered exposed to their mothers' viral genotype. All children were tested for HCV antibodies at the age of around 18 months, i.e. when passive maternal antibodies had disappeared.

The two groups of infected and uninfected children were compared with respect to the genetic variant of IL28B as well as by HCV viral genotype (HCV-gt)(Fig. 5A). The 59 mothers were also divided into two groups and labelled as transmitting or non-transmitting and compared with regard to IL28B genotype and HCV-gt (Fig. 5B). Mothers who gave birth to siblings where one was infected and the other one not, were considered as transmitting mothers.

**A**




**B**

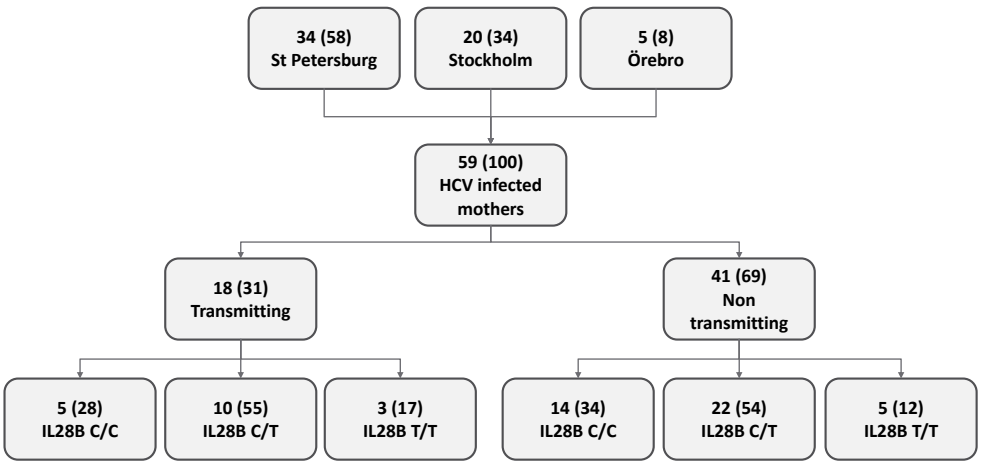



**Figure 5**. *All patients included from the three centers and subdivided by infectious status and IL28B genotype, N(%). IL28B = Interleukin 28B. A) All included children B) All included mothers*

### 4.1.4 Paper IV

This was a combined prospective and cross-sectional cohort study. Included in the study was one group of 15 HCV-RNA positive/HIV-negative mothers and their 21 children followed prospectively from birth, of whom two became infected, as well as a cross-sectional group of 13 uninfected children born to chronically HCV infected mothers and another cross-sectional group of 7 vertically HCV infected children/adolescents (Fig. 6). The cross-sectional groups of patients were added in order to increase the study population and to obtain study subjects of different ages. As adult and pediatric control groups, blood from 10 anti-HCV negative adult blood donors and cord blood from 10 newborns (born of anti-HCV negative mothers) were used. Pregnant HCV infected women were included at a maternity clinic specialized in blood borne infections in Stockholm. The cross-sectional group of uninfected children was enrolled when routinely tested for HCV antibodies at the age of 18 months. All participating mothers and children were tested at the Department of Pediatrics, Karolinska University Hospital in Stockholm.

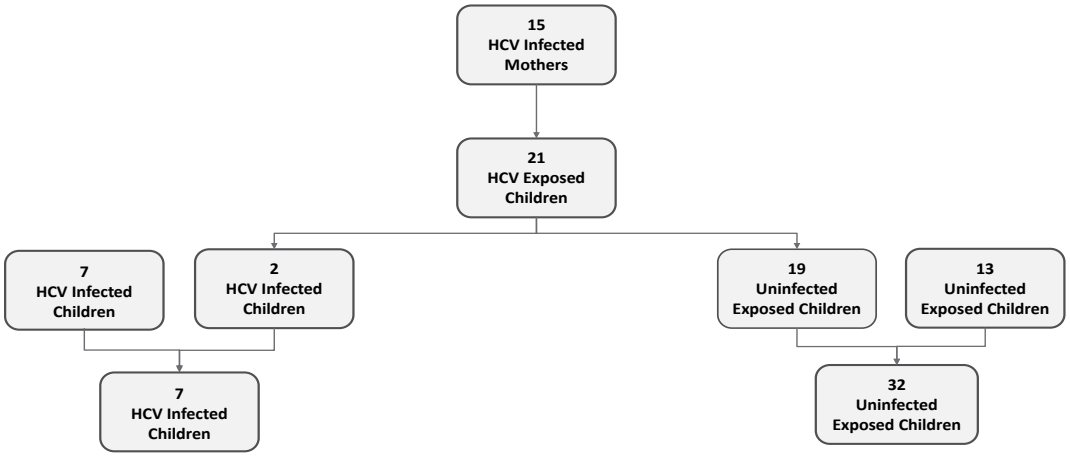



**Figure 6.** *The included mothers and children from both prospective and cross-sectional cohorts in study IV.*

Blood samples from the mothers were collected at one time point within 0-7 months post partum. In the prospectively studied group, blood samples from their children were collected consecutively at one, two or three time points at around 0, 6 and 18 months of age. In the cross-sectional groups, blood samples from the uninfected group of 13 children were collected at one time point between 18-27 months of age and samples from the group of 7 HCV vertically infected children were collected at one time point at study inclusion. Vertical infection was defined by the detection of two positive HCV-RNA blood tests with the same viral genotype as the mother within a period of at least 6 months. All blood samples were analyzed within 24 hours after venous puncture, using peripheral blood mononuclear cells (PBMC) isolated from fresh whole blood.

## 4.2 METHODS

### 4.2.1 Register analyses and medical record reviews

***Paper I.*** The patient register used in paper I was a clinical register of 255 adult patients born 1965-75 with chronic hepatitis C and registered as patients at the department of Infectious Diseases, Karolinska University hospital, Stockholm in 2005 (when the study was initiated). Their neonatal and maternity records that were mainly handwritten (not digitalized) were ordered from medical records archives from different parts of Sweden depending on residency at birth. Medical records, from the hospital's computerized record system "Take Care", were studied for the documented putative source of HCV infection at diagnosis as well as the clinical course of HCV infection.

***Paper II.*** The clinical pediatric oncology register used in this study was originally handwritten and subsequently transformed into digital form. It contained pediatric patients that had been investigated and/or treated for any form of oncological diagnosis at Karolinska university hospital, Stockholm. It included also benign diagnoses and investigated patients with suspected but not confirmed diagnoses. The small size of the register and the fact that the majority of patients it included were still alive made us hypothesize that it probably only contained patients who survived treatment passed a certain, but unknown, time point. Medical records, from the hospital's computerized record system "Take Care", were studied for documented previous anti-HCV test results. Additionally, scanned paper records in "Take Care" were analyzed. When tests were not to be found in the medical record system we then analyzed three different forms of previous (i.e. no longer in use) virological laboratory systems for occurrence of anti-HCV test in each patient. The personal ID numbers of each patient in the oncology register was crosschecked with the Swedish national public health care register of notified HCV infected patients in order to investigate how many that already had a documented HCV infection.

### 4.2.2 Virological analyses (paper II, III, IV)

***Anti-HCV***.

All anti-HCV analyses were performed at clinical routine laboratories. At Karolinska University Hospital, Division of Clinical Microbiology, the analyses were performed by third generation enzyme immuno assay (ARCHITECT Anti-HCV, Abbott, Wiesbaden, Germany).

***HCV-RNA assays***.

All HCV-RNA quantification analyses were performed at clinical routine laboratories. At Karolinska University Hospital, Division of Clinical Microbiology, the analyses were performed by TaqMan real-time PCR (Roche Molecular Diagnostics, Branchburg, NJ, USA) with a detection limit of 15 IU/ml. HCV genotyping was performed with a line probe assay (Inno-LiPA HCV II, Innogenetics NV, Gent, Belgium).

### 4.2.3 Immunological analyses (paper III and IV)

***IL28B assay***.

DNA from patients' stored plasma samples was genotyped for IL28B *rs12979860* polymorphism with TaqMan SNP genotyping assay (Applied Biosystems Inc, Foster City, CA, USA), using the ABI 7500 Fast equipment. All TaqMan probes and primers were designed and synthesised by Applied Biosystems Inc. Automated allele calling was performed using SDS software from Applied Biosystems. The primers and probes used were: *rs12979860* Forward primer: 5´GCCTGTCGTGTACTGAACCA3´, Reverse primer: 5´GCGCGGAGTGCAATTCAAC3´, Vic probe: 5´TGGTTCGCGCCTTC3´, Fam probe: 5´CTGGTTCACGCCTTC3´. Human genomic DNA was purified from 200-500 µl of plasma and preformed according to manufacturer's instructions using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), except for one change in the elution step, elution was done using 25 µl elution-buffer. The result was presented as IL28B CC, C/T or TT genotype.

***Isolation of PBMC***.

Peripheral Blood Mononuclear Cells (PBMC) were isolated from fresh whole blood (2-8 ml) using a Ficoll gradient.

***Recombinant proteins and peptides.***

Recombinant HCV proteins were pooled as NS3 and NS4 protein (3 µg/mL each), recombinant Core protein (3 µg/mL), recombinant NS5A and NS5B protein (3 µg/mL each), or a mixture of all proteins (rNS3, rNS4, rCore, rNS5A, rNS5B (3 µg/mL each)). Genotype-specific peptides covering NS3 were used and distributed in two pools with 0.6 µg/mL of each peptide (6 NS3 MHC class I peptides for genotype 1 and 3 each, and 16 NS3 MHC class II peptides for genotype 1 and 3 each). Peptides were purchased from Sigma–Aldrich (St. Louis, MO). In patients infected with or exposed to HCV genotype 2 and 4 we used peptides corresponding to genotype 3, since recombinant peptides for those genotypes were not available. All recombinant HCV proteins (Core, NS3, NS4, NS5A and NS5B) were purchased from Mikrogen (Neuried, Germany), and Concanavalin A from Sigma-Aldrich.

***ELISpot assay***.

The human IFN-γ Enzyme linked Immunospot (ELISpot) assay was performed according to the manufacturer's instruction (MabTech, Nacka Strand, Sweden) with $0.2 \times 10^6$ PBMCs/well (123). Plates were incubated for 48 hours in the presence of recombinant HCV proteins (3 µg/mL each), NS3 peptides (MCH class I and II, 0.6 µg/mL of each peptide), medium as negative control, and Concanavalin (ConA, 2 µg/mL) as positive control. Each stimulation was done in duplicates, or when enough cells were available, in triplicates and spot counts were calculated as mean spot forming units (SFU)/$10^6$ cells. A positive cut-off value for adults and children (< 2 years old) was calculated from the ELISpot results of the adult and pediatric negative control groups respectively and defined as mean SFU/$10^6$ PBMC in test wells - negative control wells + 3 x SD. The cut-off was set at > 46

SFU/$10^6$ cells for adults and > 31 SFU/$10^6$ cells for children below the age of two years. The cut-off value of > 46 SFU/$10^6$ PBMC was used for children older than 2 years (124). The positive control, ConA was > 150 SFUs/$10^6$ PBMC in all samples included in the analysis proving the viability of the cells. The number of spots was counted using the AID ELISpot reader system Version 7.0 (Autoimmun Diagnostika, Strassberg, Germany).

***3H thymidine incorporation assay***.

The HCV specific T cell proliferation was analyzed using a 3H thymidine incorporation assay. Cells were cultivated for 72 hours in 96-well U-bottom plates (Nunc) in complete cell culture medium (Sigma-Aldrich) in triplicates with $3 \times 10^5$ cells per well. After 72 h of cultivation, 3H-thymidine (Perkin Elmer) was added at a final concentration of 0.1 Ci/ml. Twenty hours later cells were harvested and 3H activity was measured by means of liquid scintillation counting (TRILUX 1450 MicroBeta counter). From the counts per minute (cpm) values, the stimulation index (SI) was calculated as a ratio of stimulated/unstimulated. A positive cut-off SI was calculated from the adult control group and defined as mean SI + 3 x SD and set at SI >2.80. Since the positive cut-off level is calculated from a quotient there was no need for a specific child control group. The adult control group was then used as comparison for both mothers and children.

***NS3 IgG antibody detection***.

The specific NS3 antibody titer from human plasma was determined by using a specific solid phase enzyme-linked immunosorbent (ELISA) assay (123). Recombinant NS3 (1 μg/mL) in 50 mM sodium carbonate buffer pH 9.6 was passively adsorbed into 96-well flat-bottom plates overnight at 4 °C. Patient plasma was serially titrated six-fold with a 1:60 starting dilution and then incubated on the plates for 1 h. Bound antibodies were detected by anti-human IgG-alkaline phosphatase antibody and p-nitrophenyl phosphate substrate tablets (both from Sigma-Aldrich, St. Louis, MO, USA). Antibody titers were determined as the last serum dilution giving an optical density at 405 nm of three times the optical density at the same dilution of plasma from a patient who has never been infected with HCV.

### 4.2.4 Statistical analyses

All statistical analyses assumed a two-sided significance level of 0.05. In the two epidemiological studies (I and II) the exact confidence interval (CI) was calculated for a binomial distribution. Bivariate analysis to compare proportions between independent groups was performed using Fisher's exact test in all studies. In study II, McNemars test was used for the analysis of the effectiveness of the two dependent screening methods. Analysis of non-parametrical data (Spot forming units and proliferation indexes for each patient) in study IV was performed using Mann-Whitney U test. Median regression analysis was used in study IV to investigate relationships between predictors and outcome.

Statistical analysis was performed with computer software Statistica (StatSoft, Tulsa, OK) in study I and with GraphPad Prism software version 6.04 and 7 (GraphPad Software, San Diego, CA) in study III-IV and STATA 13 (Stata Corporation, College Station, TX) software in study II and IV.

### 4.2.5 Ethics

All studies were performed according to the Helsinki declaration and were approved by the regional Ethics Committee.

Paper I: Dnr 269/03 + 2011/1098-32 + 2013/559-32

Paper II: Dnr 2011/1372-31/3 + 2015/1141-32 + 2017/1195-31/2

Paper III and IV: Dnr 2008/1442-31 + 2010/1263-32 + 2011/614-32 + 2012/74-32 + 2015/0081-32

# 5 RESULTS AND DISCUSSION

## 5.1 BLOOD TRANSFUSIONS AND THE RISK OF CHRONIC HCV INFECTION (PAPER I AND II)

In the first two studies (I and II) blood transfusion before 1992 as transmission route for chronic HCV infection in two different pediatric risk groups was explored.

### 5.1.1 Blood transfusion in the neonatal period and the risk of chronic HCV infection (paper I)

In study I we investigated if blood transfusion in the neonatal period could explain the transmission route in adult patients born 1960-75 with chronic HCV infection. Out of 230 patients eligible for the study, 98 (43 %) had unknown transmission route and 128 (56 %) had a documented known transmission route according to data in medical records (Fig 4). In four patients the medical records were unavailable for review. The median duration of hepatitis C infection was 32.5 years (range, 27-42 years).

**The main findings of this study were**:

I. Four out of 230 (1.7 %; 95 % CI, 0.5 % - 4.4 %) patients with chronic HCV infection had received blood product transfusion in the neonatal period. Two of them had undergone exchange transfusions.

II. None of these four patients were aware of their history of previous neonatal blood transfusions at the time of diagnosis, however one patient was soon after diagnosis found to be transfused as a neonate and was therefore regarded as a patient with known transmission route.

III. There was no significant difference in the proportion of neonatal blood transfusion recipients between those registered as having unknown (3/98, 3 %) and known (1/128, 0.8 %) transmission route ($p = 0.4$).

IV. Liver biopsies were reevaluated in three of the four patients. One of them had severe inflammation (grade 3 of 4) and liver cirrhosis (Stage IV of IV). The two other patients had mild signs of inflammation and fibrosis. Three of the four patients, including the one with cirrhosis, were successfully treated for hepatitis C.

### 5.1.2 Transfusion transmitted hepatitis C in childhood cancer survivors (paper II)

In study II we first investigated the prevalence of HCV testing and HCV infection in a group of 686 patients treated for childhood cancer in Stockholm before 1992. Out of the 686 patients, 127 had a traceable anti-HCV test. An unknown part of the cohort might have been tested outside Stockholm County and those tests were not possible to trace. The time point of testing was analyzed in order to find out how many had been tested during the national Swedish screening campaign in 2007-2010. Ten patients out of the total cohort were HCV-RNA positive with transfusion transmission as the most likely source of infection (Fig. 7).

In the second part of the study we actively traced and screened a sub-cohort of 231 presumably untested patients living in Stockholm County for HCV infection, of whom 136 accepted participation in the study. Of those, 75 took the test and 1 was found to be HCV infected (Fig. 7). In all, 11 out of 233 (5 %) tested individuals in the total cohort were found to be HCV infected.

**The main findings of this study were:**

I. The overall prevalence of chronic HCV infection among the tested childhood cancer survivors was 5 %, which is 10 times higher than the national prevalence of 0.5 % (RR=10).

II. Only 12 % of the Stockholm cohort were tested during the screening campaign in 2007-10.

III. The test uptake in the active tracing screening within this study was far better than in the national general screening campaign (39 % vs 12 %) ($p< 0.001$).

IV. In spite of long duration of HCV infection and the possible additive effect of chemotherapy, the HCV infected patients had mild to moderate signs of liver disease. The majority underwent successful antiviral treatment.

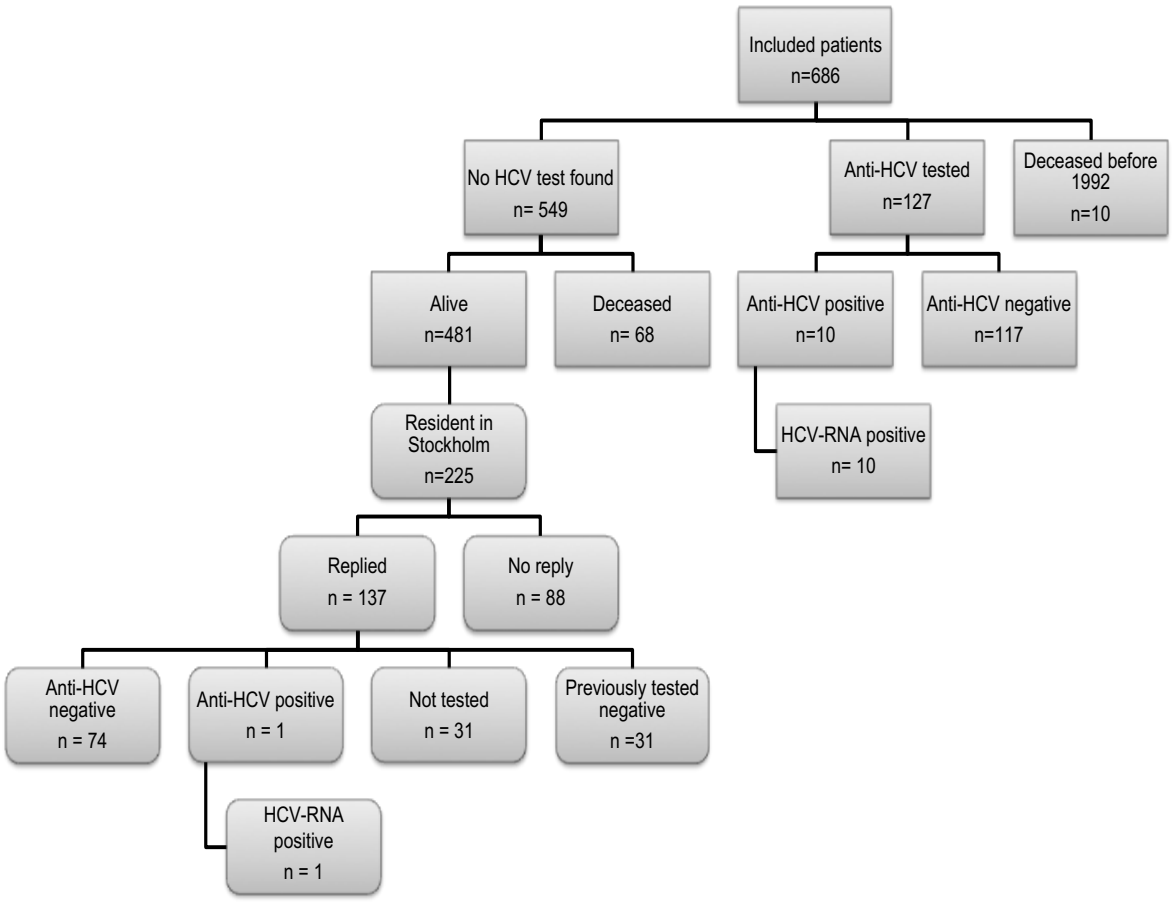



**Figure 7.** *All included patients and testing outcome in the study. Figures with rounded corners represent part 2 of the study (the active tracing screening). HCV= Hepatitis C virus*

### 5.1.3 High prevalence of chronic hepatitis C virus infection in pediatric multitransfused risk groups

Several studies have shown a high HCV prevalence among pediatric transfusion recipients in certain risk groups, for example children treated for childhood cancer, hemophilia and prematurity as well as children who underwent open-heart surgery or dialysis before 1992 (when blood donor screening was introduced) (20, 24, 28, 125, 126). Our results in study II are in line with previous findings of childhood cancer survivors where the HCV prevalence ranges between 6-40 %, depending on which malignancies that are included (25, 28). We found a relatively high prevalence of 5 % chronic HCV infection among childhood cancer survivors in Stockholm, compared to the national anti-HCV prevalence of around 0.5 %. The HCV prevalence among patients transfused at neonatal wards varies between 0.5-42 % depending on location and time period studied (18, 20). In study I we found a prevalence of 1.7 % neonatal blood transfusion recipients in a group of adult patients with chronic HCV infection.

### 5.1.4 Low awareness of previous blood transfusions in pediatric risk groups

Adult patients who received blood transfusions during childhood are often unaware of their transfusion history (127). The transfusion awareness is particularly low in individuals transfused in the neonatal period (19, 21). Furthermore, it has been shown that only 50 % of childhood cancer survivors recall previous blood transfusions during cancer treatment (23). The low awareness of transfusions given in childhood contributes to disregard of public HCV screening campaigns targeted at transfusion recipients, since they do not consider themselves as part of this risk group. If not actively traced, this large population of individuals at risk might never be tested or diagnosed.

### 5.1.5 Natural course and treatment outcome of transfusion transmitted chronic hepatitis C virus infection acquired during childhood

Individuals who were HCV infected by blood transfusions given in childhood have often a long duration of HCV infection at diagnosis. It is well known that duration of infection contributes to increased risk of developing HCV related liver cirrhosis (71). However, most studies of transfusion transmitted HCV infection acquired during childhood show that the majority has mild signs of liver disease, slow progression of liver fibrosis and a high rate of spontaneous clearance (24, 109). Apart from young age at infection, lower degree of comorbidity factors in children, such as alcohol intake, drugs and other diseases might explain this slow progression (71). A more severe liver disease, including advanced cirrhosis needing liver transplantation, has been described already during adolescence (21, 102). In study I we found that 1 out of 4 patients transfused as neonates had established liver cirrhosis at the time of diagnosis, after 27 years of infection. This patient had no other risk factors of progressive liver disease apart from the long duration of infection.

In our study (paper II) all HCV infected patients, transfused during treatment of childhood cancer, had mild to moderate liver fibrosis, albeit the long duration of infection and previous chemo- and immunosuppressive therapy, which has been shown to increase the risk of liver fibrosis (27). Our results are in line with some of the previous long-term studies on childhood cancer survivors (26, 29, 128), however Castellino et al. described a high prevalence of 13 % liver cirrhosis already after 12 years of median infection duration (27).

### 5.1.6 Hepatitis C screening of pediatric transfusion recipients

In the era of new highly effective and safe DAA treatment, the issue of finding the still undiagnosed has become crucial to achieve the goal of HCV eradication. The question of whom to screen and how to do it has drawn big attention. Several attempts to screen transfusion recipients in general look-back studies have been carried out worldwide (127, 129). The choice of screening method is a matter of time and money. Big national public screening campaigns in form of posters and public media advertisement, such as the Swedish

national screening campaign in 2007-2010, seem to be comparatively cheap and time saving, however they may fail to reach pediatric risk groups that do not recall previous blood transfusions (17). General national screening campaigns have in fact been shown to be the least cost-effective (130). In order to reach the pediatric risk groups (i.e. patients treated for prematurity, cancer or open-heart surgery) direct tracing screening is a more effective screening method, which we showed in paper II. We found that non-active tracing screening reached 12 % of patients possible to screen of whom 5 % were HCV positive and the active tracing screening reached 39 % ($p < 0.001$) of whom 1 % were HCV positive. The finding, that test uptake is better in active tracing screening, is supported by a previous meta-analysis of different screening methods (131) (Fig. 8).

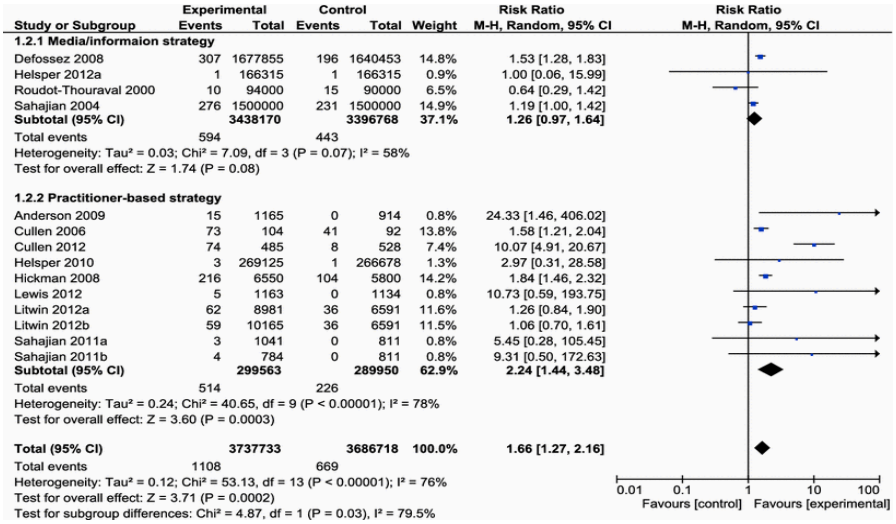



**Figure 8.** *Forest plots comparing targeted HCV testing interventions versus no targeted testing intervention by type of targeted testing: outcome; HCV antibody cases detected. From Targeted Hepatitis C antibody testing intervention: a systematic review and meta-analysis. Aspinal et al. European Journal of Epidemiology 2014. Reprinted by permission from Springer Nature.*

Patients transfused as neonates are probably least aware of their transfusion history, and for this group active tracing screening is particularly recommended. In two Swedish Counties (Halland and Västra Götaland), previous attempts to actively trace and test pediatric risk groups (as part of the national screening campaign) were made with a surprisingly low test frequency (46 % and 11 % respectively) (17). In Halland none of the 264 tested pediatric transfusion recipients were anti-HCV positive and in Västra Götaland 18 out of 479 tested were anti-HCV positive (5 previous heart surgery, 3 previous prematurity care and 10 childhood cancer survivors), of whom 14 were HCV-RNA positive (3, 3 and 8 respectively) (15, 17). Patients treated for prematurity were not possible to actively trace and test due to legal confidentiality issues and the ones found in the screening campaign had participated in the general public screening campaign. Another way to trace transfusion recipients is by so called targeted look-back studies, where tracing originates from one HCV-positive blood donor and its recipients (22, 132, 133). This option is the most time consuming one but might be highly effective since all recipients have been exposed to contaminated blood.

## 5.2 INVESTIGATION OF IMMUNOLOGICAL FACTORS IN VERTICAL TRANSMISSION OF HCV INFECTION (PAPER III AND IV)

In study III and IV the impact of certain immunological factors on vertical transmission of HCV (HCV-VT) were explored.

### 5.2.1 Lack of association between IL28B genotype and vertical transmission of hepatitis C virus (paper III)

In study III the impact of IL28B genotype on the risk of vertical HCV transmission was investigated. We included 59 mothers with chronic HCV infection and 123 children (47 infected and 76 uninfected) born of HCV infected mothers. IL28B genotype was analyzed in both groups and sorted as C/C, C/T and T/T (Fig. 5). The proportion of children with genotype C/C was the same in the vertically infected (36 %, 17/47) and the exposed uninfected children (38 %, 29/76) (Fig. 5 and Table 3 and 4).

**The main findings of this study were:**

I. Neither children's nor mother's IL28B genotype was associated with vertical transmission of HCV infection. No difference was seen when stratifying for viral genotype.

II. The IL28B genotype C/C, which has been shown to be favorable in other settings, was not protective of HCV-VT.

**Table 3.** IL28B gt distribution in children

| | C/C | Non-C/C | $P^*$ |
|---|---|---|---|
| All HCV-gt (n = 123) | | | |
| Infected (n = 47) | 17 | 30 | |
| Uninfected (n = 76) | 29 | 47 | 0.85 |
| HCV-gt 1† (n = 56) | | | |
| Infected (n = 27) | 8 | 19 | |
| Uninfected (n = 29) | 13 | 16 | 0.28 |
| HCV-gt 2† (n = 14) | | | |
| Infected (n = 3) | 2 | 1 | |
| Uninfected (n = 11) | 6 | 5 | 1.00 |
| HCV-gt 3† (n = 36) | | | |
| Infected (n = 14) | 7 | 7 | |
| Uninfected (n = 22) | 6 | 16 | 0.29 |
| HCV-gt 4† (n = 1) | | | |
| Infected (n = 0) | 0 | 0 | |
| Uninfected (n = 1) | 0 | 1 | ns |
| Unknown gt† (n = 16) | | | |
| Infected (n = 3) | 0 | 3 | |
| Uninfected (n = 13) | 4 | 9 | 0.53 |

gt = genotype; HCV = hepatitis C virus; ns = not significant.
*$P$ values calculated by Fisher exact test.
†Uninfected children were considered exposed to their mothers' viral genotype.

*IL28 genotype distribution among vertically infected vs. uninfected but exposed children, displayed as C/C or non C/C (C/T and T/T combined) for exposure to HCV genotype 1(1a+1b), 2 (2a+2b), 3 (3a + 3b), 4 and unknown genotype.*

**Table 4.** IL28B gt distribution in mothers

| | C/C | Non-C/C | $P^*$ |
|---|---|---|---|
| All HCV-gt (n = 59) | | | |
| Transmitting (n = 18) | 5 | 13 | |
| Nontransmitting (n = 41) | 14 | 27 | 0.76 |
| HCV-gt 1 (n = 28) | | | |
| Transmitting (n = 10) | 4 | 6 | |
| Nontransmitting (n = 18) | 6 | 12 | 1.00 |
| HCV-gt 2 (n = 5) | | | |
| Transmitting (n = 0) | 0 | 0 | |
| Nontransmitting (n = 5) | 1 | 4 | 1.00 |
| HCV-gt 3 (n = 24) | | | |
| Transmitting (n = 8) | 1 | 7 | |
| Nontransmitting (n = 16) | 6 | 10 | 0.35 |
| Unknown gt (n = 2) | | | |
| Transmitting (n = 0) | 0 | 0 | |
| Nontransmitting (n = 2) | 1 | 1 | 1.00 |

gt = genotype; HCV = hepatitis C virus.
*$P$ values calculated by Fischer exact test.

*IL28 genotype distribution among transmitting mothers vs. non-transmitting mothers, displayed as C/C or non C/C (C/T and T/T combined) for exposure to HCV genotype 1 (1a+1b), 2 (2a+2b), 3 (3a + 3b), 4 and unknown genotype.*

### 5.2.2 HCV specific T cell responses detected in exposed infected, and exposed but uninfected newborn children (paper IV)

In Study IV we investigated HCV specific T cell reactivity in children exposed to maternal HCV during pregnancy and delivery. All participating subjects are shown in Figure 8. In this study we showed that an HCV specific T cell response, defined as positive IFNγ response and/or HCV specific T cell proliferation, could be detected in in vitro in 73 % (11/15) of the HCV infected mothers, 67 % (6/9) of the vertically infected children, 56 % (18/32) of the exposed but uninfected children and in 10 % and 20 % of the control groups (Fig. 9).

**The main findings of this study were:**

I. The two groups of HCV exposed children both had a significantly higher proportion of HCV specific T cell responders compared to pediatric controls ($p = 0.01$ and $p = 0.02$).

II. We detected HCV specific CMI (cell mediated immune) responses in uninfected children of HCV infected mothers already in the newborn period, suggesting a pre- or perinatal exposure to viral antigens.

III. The frequency and magnitude of the CMI response in the exposed but uninfected children was similar to that detected in vertically infected children and persisted up to at least two years of age.

IV. The immune responses were mainly HCV-specific IFNγ recall responses to HCV antigens and not always linked to HCV specific T cell proliferation.

V. A significantly higher number of children in both the uninfected/exposed group and the vertically infected group had a broad IFNγ response towards multiple HCV antigen pools compared to the child controls ($p = 0.04$ and $p = 0.02$ respectively).

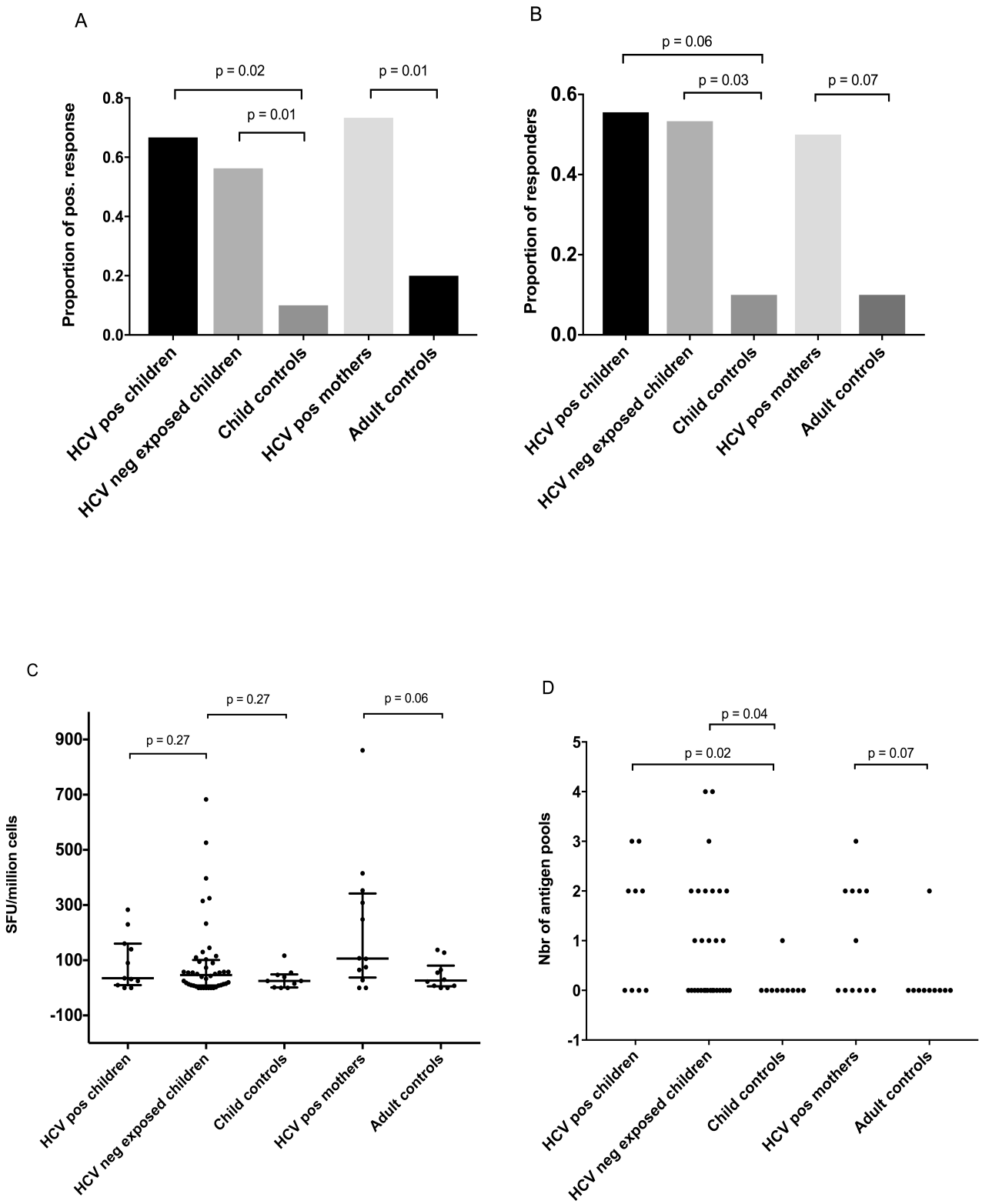



**Figure 9.** *The proportion of overall HCV specific T cell responders in the groups of patients (A), the proportion of IFNγ ELISpot responders (B), the cumulative IFNγ response (expressed as mean spot forming units (SFU)/$10^6$ cells, error bars representing median+IQR (interquartile range) (C) and the positive IFNγ response to the number of different antigen pools (D). Cut-off for a positive IFNγ response > 46 SFU/$10^6$ cells for patients > 2 years and > 31 SFU/$10^6$ cells for children < 2 years.*

### 5.2.3 The impact of IL28B genotype on vertical HCV transmission and spontaneous viral clearance (paper III)

Single nucleotide polymorphism (SNP) near the gene for IL28B (SNP *rs12979860*) was in 2009 found to be of importance to IFN based treatment outcome (64). The favorable IL28B genotype C/C was demonstrated to double the SVR rate in IFN based treatment (64). The C/C allele was also found to be three times as common in adults who spontaneously cleared HCV infection (65). IL28B genotype was later renamed Interferon-lamba-3 (IFNλ3), which is a more correct term, but for clearance reasons the term IL28B genotype is used herein since it was the term used in paper III. In paper III we investigated if IL28B genotype was associated with the risk of HCV-VT. We did not find such an association. We concluded that the, in other ways, favorable genotype C/C was not protective of HCV-VT. To the best of our knowledge, there is only one previous study similar to ours by Ruiz-Extremera et al., investigating the possible relationship between IL28B genotype and HCV-VT in 128 children. They came to the same conclusion as we did, i.e. they found no association between those two factors. However, they did find a correlation between IL28B C/C genotype and spontaneous clearance of HCV genotype 1, where 83 % of children with genotype C/C cleared the infection compared to 22 % of those with genotype non-C/C (110). This finding has been confirmed in other subsequent pediatric studies, which also conclude the same correlation independent of viral genotype (77, 107). In the largest multicenter, cross-sectional study by Indolfi et al., 177 children were included and spontaneous clearance was 2 times more likely in those with IL28B genotype C/C (79). The exact molecular mechanisms underlying the effect of IL28B genotype on spontaneous clearance and IFN treatment outcome remain unclear. IL28B induces production of IFNλ3 via the JAK-STAT pathway and presence of the unfavorable T allele results in less IFNλ3 expression and impaired host antiviral defense mechanisms (134).

### 5.2.4 Other host immune factors and vertical transmission

Since clinical factors cannot explain why HCV-VT occurs in a few but not in others, various host immunological factors have been hypothesized to play a role. Among factors studied are HLA-types, neutralizing antibodies and NK-cells. HLA-mismatch between a mother and her child has been described as favorable with regard to vertical viral transmission, since the child´s immune cells has better chance to recognize and kill the virus if its immune system is different from its mother's. HLA-mismatch and the presence of certain HLA-types have been shown to reduce the rate of vertical transmission of HCV-VT, but the findings are contradicting (43, 135, 136). It has been demonstrated that maternal neutralizing HCV-antibodies are not protective of HCV-VT (137, 138). NK-cells might be a more important player in HCV-VT than previously thought, as altered phenotype and number of NK cells has been shown to play a role in spontaneous clearance of vertically transmitted HCV infection (139). Different types of NK cell receptors; KIRs (Killer-cell immunoglobulin-like receptor) have recently been described to be associated with the rate of HCV-VT (136).

### 5.2.5 HCV specific T cell responses in children of infected mothers (paper IV)

In study IV we investigated HCV specific T cell responses in children born of HCV infected mothers. The proportion of overall T responders (defined as positive IFNγ response and/or T cell proliferation) was 73 % in the mothers, 67 % in the vertically infected children, 56 % in the uninfected/exposed children, 20 % in adult controls and 10 % in pediatric controls (Fig. 9A). The two groups of prenatally exposed children both had a significantly higher proportion of responders compared to pediatric controls ($p < 0.05$). The finding that the proportion of T cell responders was almost as high among uninfected children as in vertically infected ones is in line with the results from Della Bella et al., reporting 72 % (18/25) HCV specific T cell responders in vertically exposed/uninfected children, 67 % (2/3) of vertically infected children and 52 % (12/23) of infected mothers. However, their cohort included children age 1-4 years and thus did not give any information on the events early after birth (44). They concluded that this CMI response might be protective and contribute to the low rate of vertical HCV transmission. In a study by El-Kamary et al. a lower proportion of 14 % T cell responders was found among exposed/uninfected children (70). Babik et al. investigated the immune response to HCV exposure in utero in cord blood of children born of HCV infected mothers and found signs of general immune suppression compared to unexposed controls. On the contrary, exposed neonates had increased capacity of IFNγ production but no HCV specific T cell responses were detected (140). Studies on other HCV exposed but uninfected populations, such as household members, spouses, siblings and health care workers have reported similar findings with high proportions of HCV specific T cell responders (141-144).

The CMI response found in our study was mainly an IFNγ recall response and not always linked to T cell proliferation. On the contrary, Della Bella et al. found mainly a proliferative response whereas the IFNγ response was not significantly stronger than controls, however, they used a different method to analyze IFNγ production (43). One reason for this difference might be the choice of laboratory method to analyze HCV specific T cell proliferation. We used 3H-thymidine incorporation, a method considered golden standard, in which an isotope is incorporated within diving (proliferating) T cells upon stimulation of viral antigens in vitro. Della Bella et al used BrDU (-5-bromo-2′-deoxyuridine) incorporation flow cytometry as proliferation method. Both methods are described to correlate fairly well in terms of proliferation results (145-147). However, some drawbacks are described for 3H-thymidine incorporation, attributed to the adverse effect of β-radiation on mitotic cells (148). Another explanation might be the younger age of our study cohort, where we included some children already from 3 days of age compared to from 1 year of age in the other study. Reduced T cell proliferation and cytokine production in response to in vitro stimulation of other viral antigens has been reported in neonates (149). Since the immune system of newborns is considered immature, we calculated specific cut-off values for IFNγ ELISpot in children younger than 2 years of age based on the mean spot-forming unit (SFU) of the cord blood controls + 3 SD. The cut-off SFU for children < 2 years was set at $31/10^6$ cells and at $46/10^6$

cells for adults and children > 2 years (based on the results of the adult control group). Lack of standardized cut-off ELISpot SFU values for children lead to a wide range of test results reported in studies. The cut-off value used in the study by El-Kamary et al. was > 55 SFU/$10^6$ cells (70).

### 5.2.6 The timing of vertical transmission and early cell mediated immune responses (paper IV)

We detected an HCV specific T cell (IFNγ) response towards viral antigens in vitro in some uninfected children already in the newborn period suggesting a pre- or perinatal exposure to viral antigens. As many as 60 % of the exposed/uninfected children had an IFNγ response before 3 months of age. In utero exposure to viral antigens might therefore be more common than previously thought. It is unclear exactly when vertical transmission occurs, but based on when HCV-RNA is first detected in newborn children it is thought that approximately one third is infected during pregnancy and the rest during delivery (34, 150, 151).

### 5.2.7 Is HCV specific T cell reactivity protective of infection? (paper IV)

One could speculate if exposure to viral antigens during pregnancy and subsequent development of HCV specific T cell reactivity contributes to some sort of protective immunity against vertical HCV infection. If this were true, we would have expected uninfected children to have a higher proportion of T cell responders than the vertically infected children. However, the proportion of T cell responders was almost equal in the two groups (67 % of the infected and 56 % of the uninfected) (Fig. 9A), suggesting that an HCV specific T cell response alone is not enough to protect from vertical infection. However, the vertically infected children were older and their T cell responses could have developed as a consequence of their chronic HCV infection, as shown in the chronically infected mothers.

In chimpanzees, it has been demonstrated that HCV specific memory T cell responses are protective and associated with rapid virological control and clearance of HCV infection within 14 days when reinfected 7 years later (152). Similar results were found in another chimpanzee study where rechallenge took place 16 years after primary infection (68). In humans, reinfection challenge studies are not possible but by prospectively following individuals at high risk of reinfection, HCV specific immune responses and infection outcome can be investigated. Protective, but not sterilizing, immunity has been reported in IDUs, where reinfection yielded a shorter viremia period with lower HCV-RNA levels associated with broad IFNγ recall T cell responses (63). The rate of protective immunity might be underestimated, since a rapid clearance of reinfection might be missed if blood-sampling frequency is too low. Others have confirmed these findings in similar settings, concluding that protective immunity depends on the breadth, quality and magnitude of HCV specific memory T cell responses (66).

# 6 CONCLUSIONS AND FUTURE PERSPECTIVES

Vertical transmission of hepatitis C virus is the most common transmission route in children. Even though our understanding of involved host and viral factors has increased, there is today no intervention to prevent HCV-VT. The only way to eliminate this risk is to eradicate the infection in fertile women, which is possible with the new treatment options. The main obstacle is to find the still undiagnosed, which puts new light on the importance of HCV screening of risk groups. The aim of this thesis was to investigate the transmission routes of HCV infection in children and below are the main conclusions described.

- In study I we found that blood transfusions in the neonatal period before 1992 constitutes the transmission route in approximately 1.7 % of a cohort of chronic HCV patients in Stockholm. The finding of low awareness of previous blood transfusions and few clinical symptoms of chronic HCV in combination with the finding of liver cirrhosis in one of the four patients, suggests that efforts should be made to actively trace and screen this risk group for HCV.

  Patients treated at neonatal wards were pointed out as a special pediatric risk group in the national screening campaign in 2007-2010 but data analysis from the tested population shows that the campaign failed to reach this group. One explanation for this is probably the low awareness of previous hospital admittance in the neonatal period. Active tracing and testing of this risk group is thus needed. The best way would be to target only those with documented neonatal blood transfusions before 1992, however, one obstacle might be the lack of digitalized transfusion records from that time.

- In study II we found a 10 times higher prevalence of chronic HCV infection among childhood cancer survivors in Stockholm compared to the general Swedish population. Active tracing screening was a better screening method than the general public screening campaign. The majority underwent successful antiviral treatment. In the era of highly effective DAA treatment, we recommend active tracing screening of all childhood cancer survivors.

- In study III we found no association of IL28B genotype and risk of vertical transmission. We conclude that, the in other settings favorable genotype C/C, is not protective of vertical transmission. The reason for the low vertical transmission rate of HCV remains unknown. Future studies on other possible host or viral factors involved in vertical HCV transmission are needed, for example exploring the role of NK cells and viral diversity.

- In study IV we found a high proportion of HCV specific T cell responders among exposed/uninfected children already from the newborn period, suggesting a prenatal exposure to viral antigens. The immune response persists up to least two years of age. HCV specific T cell response is a sign of previous viral encounter in the absence of

HCV antibodies. The frequency or magnitude of the CMI response was not higher than in vertically infected children, suggesting that CMI responses alone are not protective of vertical transmission. However, the T cell response in the vertically infected children might have developed as a consequence of their chronic HCV infection since they were older than the exposed/uninfected group.

Future larger prospective studies on newborn children exposed to maternal HCV during pregnancy could better determine if a strong HCV specific T cell response early in life is associated with a lower risk of vertically transmitted chronic HCV infection. The results of the studies on HCV specific immune responses might be useful if the need for an HCV vaccine turns out to be necessary in the future, given for example possible DAA resistance development.

# 7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Hepatit C är ett virus som infekterar levern. Det är en blodburen smitta som oftast blir kronisk och kan leda till utveckling av leverfibros, skrumplever och cancer. Hepatit C virus (HCV) infektionen är nästan alltid symtomfri, varför många bär på infektionen utan att veta om det. WHO har uppskattat att ca 6 miljoner barn världen över är HCV infekterade. Den vanligaste smittvägen för barn idag är smitta från modern under graviditet och förlossning. Tidigare var även blodtransfusion med smittat blod en vanlig smittkälla. År 1992 infördes allmän blodgivarscreening i Sverige för hepatit C, vilket medfört att den risken idag är obefintlig. Sedan några år tillbaka finns en mycket effektiv och nästan biverkningsfri tablettbaserad behandling mot hepatit C som ger nästintill 100 % utläkning. Denna behandling är idag godkänd för barn över 12 år. För att kunna minska fortsatt spridning av viruset och därmed minska risken för leversjukdom är det viktigt att vi hittar alla de som fortfarande är odiagnostiserade. Syftet med denna avhandling har varit att studera smittvägar samt epidemiologiska och immunologiska aspekter av hepatit C exponering tidigt i livet.

I **studie I** undersöktes om blodtransfusioner givna under nyföddhetsperioden kunde förklara smittvägen hos de med kronisk HCV infektion där smittvägen var okänd. Vi inkluderade 230 patienter födda 1960-75 med kronisk HCV infektion och studerade deras journaler. Av dessa, hade 98 okänd smittväg och 128 känd smittväg. Fyra av 230 (1.7 %) hade fått blodtransfusion som nyfödda och detta var den mest sannolika smittvägen i samtliga fall. Ingen av dem var, vid tiden för hepatit C diagnosen, medvetna om att de fått blod i nyföddhetsperioden. Tre av dem genomgick behandling och läkte ut sin hepatit C.

I **studie II** undersöktes 676 nu vuxna patienter som under barndomen behandlats för cancer i Stockholm före 1992 (när blodgivarscreening infördes). Blodtransfusioner är vanliga vid cancerbehandling och dessa patienter räknas därför som särskild riskgrupp för transfusionsöverförd HCV smitta. Vi undersökte med hjälp av journaler och register hur många som var HCV testade och hur många som var HCV infekterade. Vi fann att 233 av 676 (34 %) var HCV testade, och 11 av dem (5 %) var infekterade. Andelen infekterade i denna grupp patienter visade sig vara 10 gånger högre än i den svenska befolkningen. De patienter som år 2017 bodde i Stockholms län och sannolikt var otestade inkluderades i en screening där vi aktivt sökte upp och erbjöd dessa patienter HCV test. Denna screeningstrategi visade sig vara betydligt mer effektiv avseende antalet testade jämfört med en tidigare nationell HCV screeningkampanj där alla som fått blod före 1992 via media uppmanades gå och testa sig.

I **studie III** undersöktes om en genetisk variant av en immunsignalmolekyl, kallad IL28B, påverkar risken att en mor smittar sitt barn under graviditet eller förlossning (s.k. vertikal smitta). Denna risk beräknas generellt vara ca 5 % och är oberoende av förlossningssätt och amning. Däremot ger hög virusnivå hos modern ökad risk att barnet blir smittat. IL28B genotyp har i tidigare studier visat sig ha betydelse för spontan utläkning av kronisk HCV infektion samt för utläkningsfrekvensen vid interferonbaserad behandling (den äldre typen av behandling). Vi inkluderade 59 mödrar med kronisk HCV infektion och 123 barn till

infekterade mödrar, varav 47 infekterade och 76 oinfekterade. Varken barnens eller mödrarnas IL28B genotyp visade sig ha någon betydelse för smittöverföringsrisken.

I **studie IV** studerades HCV specifikt immunsvar (T cellssvar) hos barn till HCV infekterade mödrar. Förekomsten av virusspecifikt T cellssvar är ett tecken på immunologiskt minne, dvs att T cellerna tidigare har stött på samma viruspartikel. T cellssvaret hos barn och mammor undersöktes med två olika labmetoder som dels mäter hur mycket gammainterferon (signalmolekyl med antiviral verkan) T cellerna producerar, dels hur snabbt de delar sig när de utsätts för HCV proteiner. Vi kunde påvisa ett HCV specifikt T cellssvar hos 73 % av mödrarna, hos 67 % av infekterade barn och hos 56 % av de exponerade men oinfekterade barnen. T cellsvaret fanns redan hos nyfödda barn vilket indikerar att de har exponerats för viruspartiklar redan under graviditeten (eftersom det tar viss tid för ett T cellssvar att utvecklas). Vi kunde inte påvisa någon signifikant skillnad mellan infekterade och exponerade/oinfekterade barn, däremot hade båda exponerade barngrupperna högre andel med T cellssvar än friska nyfödda barn till osmittade mammor. I gruppen smittade barn ingick barn i åldrarna 0-18 år och i exponerade/oinfekterade gruppen 0-2 år. Det faktum att så många osmittade men HCV exponerade barn hade ett HCV specifikt T cellssvar tyder på att virusexponering under graviditet och förlossning kan vara vanligare än man tidigare trott. Det är möjligt att detta svar bidrar med någon form av medfött immunologiskt skydd mot vertikalsmitta. För att kunna fastställa om T cellssvaret har någon skyddande effekt behövs fler liknande studier med tillräckligt många nyföddhetsprover av både oinfekterade och infekterade HCV exponerade barn.

Sammanfattningsvis, mot bakgrund av våra resultat där vi fann låg medvetenhet och låg andel HCV testade i kombination med hög andel smittade, rekommenderas aktiv spårning och testning av personer tillhörande riskgrupper för HCV transfusionssmitta. Vi fann inget samband mellan IL28B genotyp och mor-barn smitta, men däremot en hög andel exponerade men oinfekterade barn som hade HCV specifikt T cellssvar redan i nyföddhetsperioden vilket indikerar att exponering för viruspartiklar under graviditeten sannolikt är vanligare än man tidigare trott.

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